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硕 士 学 位 论 文

饲料蛋白质水平对卵形鲳鲹消化酶、非特异性免疫和肠道内容物细菌的影响

Effects of dietary protein levels on digestive enzymes, non-specific immunity and intestinal contents bacteria of *Trachinotus ovatus* (Linn)

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缩略词表

缩略词	英文全称	中文全称
ACP	Acid Phosphatase	酸性磷酸酶
AKP	Alkaline Phosphatase	碱性磷酸酶
AMS	Amylase	淀粉酶
APS	Ammonium Persulfate	过硫酸铵
ATP	Adenosine Triphosphate	三磷酸腺苷
CAT	Catalase	过氧化氢酶
CCK	Cholecystokinin	肠促胰酶肽
DGGE	Denaturing Gradient Gel Electrophoresisi	变形梯度凝胶电泳
DHA	Docosahexaenoic acid	二十二碳六烯酸
EAA	Essential Amino Acid	必需氨基酸
EPA	Eicosapentaenoic Acid	二十碳五烯酸
FAO	Food and Agriculture Organization of the United Nations	联合国粮农组织
FISH	Fluorescence In Site Hybridizatioin	荧光原位杂交
GPx	Glutathione Peroxidase	谷胱甘肽过氧化物酶
GSH	Glutathione	还原型谷胱甘肽
GSSG	Oxidized Glutathione	氧化型谷胱甘肽
LPS	Lipase	脂肪酶
LYZ	Lysozyme	溶菌酶
MDA	Malondialdehyde	丙二醛
MOS	Mannan oligosaccharides	甘露寡糖
NEAA	Non-essential Amino Acid	非必需氨基酸
NO	Nitric Oxide	一氧化氮
PBS	Phosphate Buffer Solution	磷酸盐缓冲液
PCR	Polymerase Chain Reaction	聚合酶链式反应

缩略词表(续)

PUFA	Polyunsaturated Fatty Acid	多不饱脂肪酸
rDNA	Ribosome Deoxyribonucleic Acid	核糖体脱氧核糖核酸
ROS	Reactive Oxygen Species	氧自由基
SDS	Sodium Dodecyl Sulfate	十二烷基硫酸钠
SOD	Superoxide Dismutase	超氧化物歧化酶
TAE	Tris-Acetic Acid Electrophoretic Buffer	Tris-乙酸电泳缓冲液
T-AOC	Total Antioxidant Capacity	总抗氧化能力
TEMED	N,N,N',N'-Tetramethylethylenediamine	N, N, N', N'-四甲基二乙胺
TGGE	Temperature Gradient Gel Electrophoresisi	变性温度凝胶电泳
TRY	Trypsin	胰蛋白酶
V_A	Vitamin A	维生素 A
V_C	Vitamin C	维生素 C
V_D	Vitamin D	维生素 D
V_E	Vitamin E	维生素 E
3, 5-DNS	3, 5-Dinitrosalicylic Acid	3, 5 二硝基水杨酸

摘 要

以初始体重为(518.81±15.99) g 的卵形鲳鲹(*Trachinotus ovatus*, Linn)为实验对象，鱼粉、豆粕为主要蛋白源，鱼油为脂肪源，配制蛋白质水平分别为 38%，41%和 44%，脂肪水平均为 8%的 3 种等脂等能的饲料投喂卵形鲳鲹，处理组依次记为 A 组(38%)、B 组(41%)、C 组(44%)，进行为期 30 d 的饲养试验，探讨饲料蛋白质水平对卵形鲳鲹肌肉营养成分、消化酶活性、非特异性免疫指标以及肠道内容物细菌的影响。主要研究结果如下：

1. 饲料蛋白质水平对卵形鲳鲹肌肉营养成分的影响

饲料蛋白质水平对卵形鲳鲹肌肉中蛋白质、氨基酸、脂肪和脂肪酸含量总体上影响不大。随着饲料蛋白质水平的升高，卵形鲳鲹肌肉中蛋白质、氨基酸含量表现出升高趋势，但升幅不大，各处理组卵形鲳鲹肌肉蛋白质含量在 19.6-20.0 %之间波动，总氨基酸含量在 187-204 g/kg 之间；脂肪和脂肪酸含量随饲料蛋白质水平升高变化不明显，各处理组卵形鲳鲹肌肉脂肪含量在 11.0-12.2%间波动，饱和脂肪酸和不饱和脂肪酸含量分别为 4.1-4.5%，6.9-7.7%。

2. 饲料蛋白质水平对卵形鲳鲹消化酶活性的影响

饲料蛋白质水平对卵形鲳鲹肝脏、肠道中胰蛋白酶(TRY)和胃蛋白酶(Pepsin)活性影响显著($P<0.05$)，各组织器官中胰蛋白酶和胃蛋白酶活性变化范围分别为 584.90-9185.84 U/mg prot、4.79-42.34 U/mg prot；对脂肪酶(LPS)、淀粉酶(AMS)及纤维素酶(Cellulose)活性影响不显著($P>0.05$)，脂肪酶、淀粉酶和纤维素酶活性分别在 37.97-312.35 U/mg prot、0.15-0.51 U/mg prot 和 0.25-9.13 U/mg prot 之间变化。卵形鲳鲹消化酶活性具有显著的组织差异性($P<0.05$)，除纤维素酶外，其他消化酶活性均表现为前肠>中肠>后肠>肝脏。说明前肠是卵形鲳鲹消化蛋白质营养的主要部位，提高饲料蛋白质水平有利于卵形鲳鲹对蛋白质的消化利用。

3. 饲料蛋白质水平对卵形鲳鲹非特异性免疫的影响

随着饲料蛋白质水平升高，各处理组卵形鲳鲹血清溶菌酶(LYZ)活性先显著升高后显著降低($P<0.05$)，LYZ 活性最高出现在 B 组，为 17.65 U/mg prot；各组织器官中碱性磷酸酶(AKP)、酸性磷酸酶(ACP)和谷胱甘肽过氧化物酶(GPx)活性先显著升高后显著降低($P<0.05$)，B 组肠道中 AKP 和 ACP 活性最高，分别为

10056.14 U/mg prot、6633.06 U/mg prot， GPx 活性最高出现在 B 组肝脏，为 593.87U/mg prot；SOD 和 CAT 活性显著升高(P<0.05)，最高值均出现在 C 组肝脏，分别为 332.16U/mg prot 和 42.18U/mg prot；总抗氧化能力(T-AOC)显著增强(P<0.05)，肝脏 T-AOC 最强，最高值为 78.40 单位/mg prot；丙二醛(MDA)含量显著下降(P<0.05)，C 组肌肉 MDA 含量最低，为 0.48 nmol/mg prot。综合各非特异性免疫指标的变化结果发现，B 组卵形鲳鲹免疫能力较强，即当饲料蛋白质水平为 41%时，对卵形鲳鲹生长健康较为有利。

4. 饲料蛋白质水平对卵形鲳鲹肠道内容物细菌的影响

PCR-DGGE 指纹图谱分析表明，B 组卵形鲳鲹后肠内容物细菌种类最多，多样性最高，其肠道内容物细菌 16S rDNA V3 区基因通过 DGGE 分离后得到 24 条位置各异的条带，多样性指数为 3.156。B 组卵形鲳鲹前肠内容物细菌种类最少，多样性最低，其肠道内容物细菌 16S rDNA V3 区基因通过 DGGE 分离后得到 19 条位置各异的条带，多样性指数为 2.732。各样品肠道内容细菌群落的相似性分析表明，C 组卵形鲳鲹中肠和后肠肠道内容物细菌群落相似性最高，相似性系数为 50.00%，B 组中肠和 C 组中肠肠道内容物细菌群落相似性最低，相似性系数为 37.14%。各样品聚类分析表明，C 组前、中和后肠的聚类位置最分散，即 C 组卵形鲳鲹前、中和后肠道内容物细菌群落组成差异最大，说明饲料蛋白质水平为 44%时，对卵形鲳鲹肠道内容物细菌群落结构的影响较大。综合以上结果表明，饲料蛋白质水平会对卵形鲳鲹肠道内容物细菌群落组成造成影响。优势条带回收测序结果表明，卵形鲳鲹肠道内容物内的优势菌群主要属于变形菌门(Proteobacteria)、厚壁菌门(Firmicutes)和绿弯菌门(Chloroflexi)。

关键词：卵形鲳鲹；饲料蛋白质水平；消化酶；非特异性免疫；肠道内容物细菌

Abstract

A 30-day feeding trail was conducted to evaluate effects of dietary protein levels on body composition, digestive enzyme activities, non-specific immunity parameters and intestinal bacteria of golden pompano (*Trachinotus ovatus*, Linn). Three isoenergetic and isolipid (8%) experimental diets with fish meal and soybean meal as protein source and fish oil as lipid source were formulated to contain different protein levels (38%, 41% and 44%). Each diet was randomly fed to triplicate groups of fish with initial weight of 518.81±15.99g. Fish fed three graded diets were labeled as group A, B and C. The main results are as follows:

1. Effects of dietary protein levels on body composition of *Trachinotus ovatus*

The contents of the protein, amino acids, lipid and lipid acids in muscle of *Trachinotus ovatus* were not influenced by the dietary protein levels. With the increasing of deitary protein levels, contents of muscular protein and amino acids showed slightly increasing tendencies. The protein and total amino acids contents of muscle of each group fluctuated between 19.6-20.0 % and 187-204 g/kg respectively. Contents of muscular lipid, saturated fatty acids and unsaturated fatty acids slightly varied from 11.0 to 12.2%, 4.1 to 4.5% and 6.9 to 7.7% respectively with increasing dietary protein levels.

2. Effects of dietary protein levels on digestive enzyme of *Trachinotus ovatus*

Dietary protein levels significantly influenced the hepatic and intestinal trypsin and pepsin activities ($P<0.05$) of *Trachinotus ovatus*. The variation ranges of these two proteolytic enzymes in intestinal, gill, liver and muscle were 584.90-9185.84 U/mg prot and 4.79-42.34 U/mg prot respectively. Dietary protein levels has no significant effects on activities of lipase, amylase and cellulose of intestinal, gill, liver and muscle of *Trachinotus ovatus* ($P<0.05$). Activities of lipase, amylase and cellulose of tissues and organs varied from 37.97 to 312.35 U/mg prot, 0.15 to 0.51 U/mg prot and 0.25 to 9.13 U/mg prot respectively. In this study, the activities of all digestive enzymes had significant differences among different tissues and organs ($P<0.05$). Digestive enzymes activities of *Trachinotus ovatus* showed

foregut>midgut>hindgut>liver except cellulose. Results indicated that foregut was the primary region of dietary protein digestion in *Trachinotus ovatus* digestive tract. Increasing the level of dietary protein would facilitate the protein digestive ability of *Trachinotus ovatus*.

3. Effects of dietary protein levels on the parameters of non-specific immunity of *Trachinotus ovatus*

Serum lysozyme activities of *Trachinotus ovatus* of three groups were significantly increased and then decreased with the increase of dietary protein levels ($P<0.05$). Highest lysozyme activity which occurred in group B was 17.65 U/mg prot. Alkaline phosphatase (AKP), acid phosphatase (ACP) and glutathione peroxidase (GPx) of all tissues and organs in *Trachinotus ovatus* were significantly increased and then decreased with increasing dietary protein levels ($P<0.05$). Highest AKP and ACP activities occurred in intestine of *Trachinotus ovatus* in group B were 10056.14 U/mg prot and 6633.06 U/mg prot. Highest GPx activity occurred in liver of *Trachinotus ovatus* in group B was 593.87 U/mg prot. The activities of superoxide dismutase (SOD) and catalase (CAT) and total antioxidant capacity (T-AOC) were significantly increased with increasing dietary protein levels ($P<0.05$). The highest SOD and CAT activities and T-AOC in liver were 332.16 U /mg prot, 42.18 U /mg prot and 78.40 U/mg prot respectively. Malondialdehyde (MDA) contents in all tissues and organs significantly negatively correlated with increasing dietary protein levels ($P<0.05$). The malondialdehyde content in muscle of *Trachinotus ovatus* in group B was the lowest with a value of 0.48 nmol/mg prot. The results indicated that the non-specific immunity would be enhanced when fed *Trachinotus ovatus* with dietary protein 41%.

4. Effects of dietary protein levels on bacteria in intestinal contents of *Trachinotus ovatus*

PCR-DGGE, a technology of molecular ecology, was used to investigated differences of bacteria in intestinal contents of *Trachinotus ovatus* fed dietaries with different protein levels. Analysis of PCR-DGGE fingerprint indicated that bacteria community in hindgut contents of *Trachinotus ovatus* in group B had the highest diversity with a value of 3.156. Bacteria community of foregut contents of

Trachinotus ovatus in group B had the lowest diversity with a diverse value of 2.732. Similarity analysis indicated that bacteria community of midgut and hindgut of *Trachinotus ovatus* in group C were the most similar with a similarity coefficient of 50%. Bacteria communities of midgut of *Trachinotus ovatus* in group B and group C were the most different with a similarity coefficient of 37.14%. The clustering analysis indicated that bacteria communities of different intestinal segments of *Trachinotus ovatus* in group C showed furthest distances which meant diet contain 44% protein influenced the intestinal bacteria community structure of *Trachinotus ovatus* mostly. The sequencing results of bands cut from DGGE gels showed that dominant bacteria of *Trachinotus ovatus* intestine mainly belonged to Proteobacteria, Firmicutes and Chloroflexi.

Key Words: *Trachinotus ovatus*; Dietary protein levels; Digestive enzyme; Non-specific immunity; Intestinal bacteria.